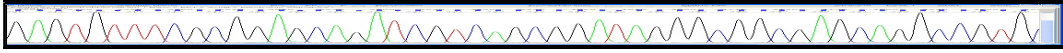


Genetic Research Services

DNA Sequencing Service Reaction Guide



Recommended Amounts Of Template For Sequencing Reactions

Template	Quantity
PCR Product: 100-200bp 200-500bp 500-1000bp 1000-2000bp >2000bp	1-3ng 3-10ng 5-20ng 10-40ng 20-50ng
Single-Stranded	25-50ng
Double-Stranded	150-300ng
Cosmid, BAC	0.5-1.0µg
Bacterial Genomic DNA	2-3µg

PRIMER: 3.2pmol

We **strongly** recommend running a small amount of your DNA (either PCR product or plasmid) on a gel to quantify the amount of DNA template.

Reaction Setup

BigDye	1:1	1:2	1:4	1:8	1:16	1:32
	8	4	2	1	0.5	0.25
5xSB	0	2	3	3.5	3.75	3.875
Primer						
Template	12	14	15	15.5	15.75	15.875
H₂O						
Total	20	20	20	20	20	20

Note that shorter templates (eg PCR products) require less Big Dye terminator

Cycling Conditions

94°C for 5 min
 96°C for 10 sec
 50°C for 5 sec
 60°C for 4 min
 Hold at 4°C

} x20-30 cycles